PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 42:e07166, 2022
DOI: 10.1590/1678-5150-PVB-7166

Original Article
Wildlife Medicine

Type C botulism outbreak in free-ranging waterfowl in Goiás

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ABSTRACT.- Martins A.S., Silva T.A., Rosa I.C.C.R., Oliveira A.G.F., Oliveira M.P., Saraiva J.R., Dutra I.S. & Borsanelli A.C. 2022. **Type C botulism outbreak in free-ranging waterfowl in Goiás.** *Pesquisa Veterinária Brasileira 42:e07166, 2022*. Departamento de Medicina Veterinária, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Rodovia Goiânia Km 8, Nova Veneza, Goiânia, GO 74690-900, Brazil. E-mail: anaborsanelli@ufg.br

Botulism is generally a fatal disease caused by ingestion of neurotoxins produced by *Clostridium botulinum.* The present study describes the epidemiological, clinical, and laboratory aspects of a type C botulism outbreak in free-living aquatic birds residing in an urban park in Quirinópolis, Goiás, Brazil. Among a population of approximately 80 waterfowl, a total of 30 birds, including ducks (*Cairina moschata*), teals (*Anas platyrhynchos*), and geese (*Anser cygnoides*), died within 10 days. Of these, six birds showed signs of flaccid paralysis of the pelvic limbs, eyelids, neck, and wings. To confirm the suspicion of botulism, four lake water samples, two samples of the feed consumed by the birds, and samples of serum, intestinal content, stomach content, and liver tissue from two teals that died after presenting clinical signs were analyzed. Using bioassay and neutralization with homologous antitoxin in mice, it was possible to detect the presence of botulinum toxin type C in a water sample and in the intestinal content of one of the necropsied teals. Additionally, the presence of *C. botulinum* type C was identified in the lake water using polymerase chain reaction. Based on the clinical signs and laboratory results, a diagnosis of botulism caused by botulinum toxin type C was confirmed with probable transmission by lake water.

INDEX TERMS: *Clostridium botulinum*, flaccid paralysis, avian botulism, botulinum toxins, birds, waterfowl, Brazil.

RESUMO.- [**Surto de botulismo tipo C em aves aquáticas de vida livre em Goiás.**] O botulismo é uma doença geralmente fatal, causada pela ingestão de neurotoxinas produzidas pelo *Clostridium botulinum.* O presente estudo descreve os aspectos epidemiológicos, clínicos e laboratoriais de um surto de botulismo tipo C em aves aquáticas de vida livre habitantes de parque urbano em Quirinópolis, Goiás. De uma população de cerca de 80 aves aquáticas, um total de 30 aves, entre patos (*Cairina moschata*), marrecos (*Anas platyrhynchos*) e gansos (*Anser cygnoides*), morreram no intervalo de 10 dias. Destes, seis aves apresentaram sinais de paralisia flácida de membros pélvicos, pálpebras, pescoço e asas. Para confirmar a suspeita de botulismo, foram analisadas quatro amostras da água do lago, duas amostras da ração consumida pelas aves e amostras de soro, conteúdo intestinal, conteúdo estomacal e fígado de dois marrecos que morreram após apresentarem os sinais clínicos. Pelo bioensaio e neutralização com antitoxina homóloga em camundongos foi possível detectar a presença de toxina botulínica tipo C em uma amostra de água e no conteúdo intestinal de um dos marrecos necropsiados. Adicionalmente, pela reação em cadeia da polimerase identificou-se a presença de *C. botulinum* tipo C na água do lago. Com base nos sinais clínicos e resultados laboratoriais estabeleceu-se o diagnóstico de botulismo causado pela toxina botulínica tipo C e veiculada provavelmente pela água do lago.

TERMOS DE INDEXAÇÃO: Botulismo, surto, aves aquáticas, *Clostridium botulinum*, Brasil.

[1] Received on August 26, 2022.
Accepted for publication on September 12, 2022.
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INTRODUCTION

Surveillance and monitoring of mortality events in free-ranging animals are important tools for elucidating and preventing epizootic outbreaks (Morner et al. 2002). Botulism is a disease of great relevance in waterfowl worldwide and has the potential to cause a decline in the population of several species due to its high lethality (Rocke 2006). Outbreaks of avian botulism are associated with ingestion of preformed botulinum neurotoxins in decaying organic matter, stagnant water, and fly larvae and other invertebrates present in carcasses (Rocke & Bollinger 2007).

Clostridium botulinum is a sporulating and putrefactive anaerobic microorganism that is widely distributed in nature, soil, and sediments of lakes and seas. Under anaerobic conditions, spores can germinate, transform into a vegetative form, and produce neurotoxins (Scarcelli & Piatti 2002). To date, eight different botulinum neurotoxin serotypes with similar pharmacological actions have been identified and classified in A, B (Burke 1919), C (Bengston 1922), D (Gunnison & Meyer 1929), E (Gunnison et al. 1936), F (Moller & Scheibel 1960), G (Gimenez & Ciccareli 1970) and H (Barash & Arnon 2014). Regarding birds, serotypes C and C/D are the most reported in outbreaks, although types E and A can also be associated with botulism outbreaks in birds (Linares et al. 1994, Neimanis et al. 2007, Chipault et al. 2015, Badagliacca et al. 2018, Lima et al. 2020, Rosciano et al. 2021, Rogers et al. 2021, Quevedo et al. 2022).

When ingested, botulinum toxin is absorbed in the gastrointestinal tract and spreads to the presynaptic membrane of the neuromuscular junction, blocking the release of acetylcholine and resulting in transmission failure of nerve impulses at the nerve fiber junctions, leading to the absence of acetylcholine, muscle contraction, and consequent flaccid paralysis. The speed and severity of clinical signs are proportional to the amount of toxin ingested. Affected birds show signs of progressive weakness, motor incoordination, paresis, flaccid paralysis of skeletal muscles, and inability to fly, walk, and keep the head upright (Trampel et al. 2005).

In Brazil, there are few reports on the occurrence of botulism in free-ranging waterfowl. Thus, the present study aimed to describe a type C botulism outbreak in a population of free-living waterfowl, including ducks (*Cairina moschata*), teals (*Anas platyrhynchos*), and geese (*Anser cygnoides*), in the municipality of Quirinópolis, Goiás, Brazil.

MATERIALS AND METHODS

Outbreak description. An outbreak of botulism in free-living waterfowl occurred in the lake region of Parque da Liberdade (longitude UTM: 559768.00; latitude UTM: 7959439.00), an urban park of approximately five hectares located in the municipality of Quirinópolis, Goiás, Brazil (Fig.1). The municipality has an area of

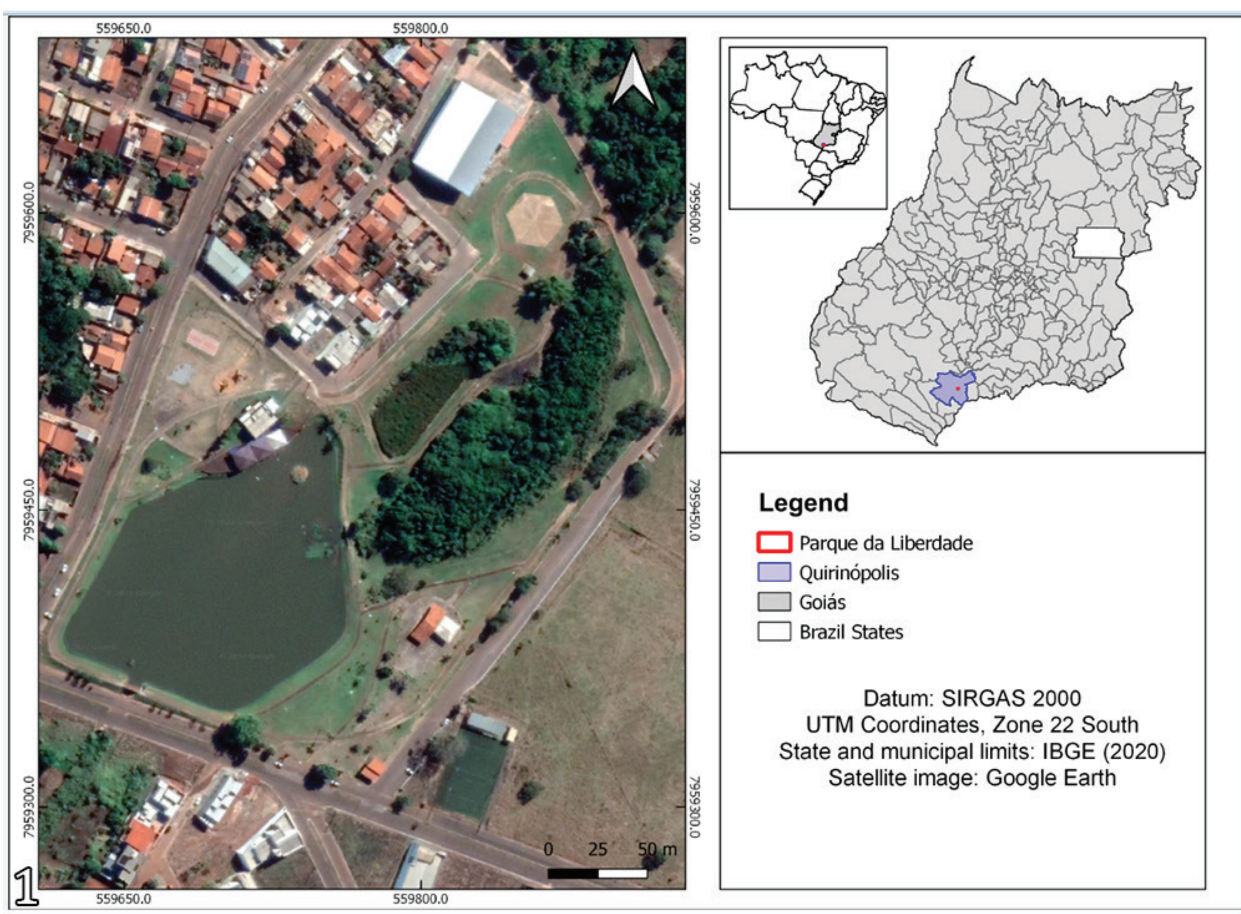

Fig.1. Parque da Liberdade location map. On the left, a detailed view of the urban park where the outbreak of botulism in waterfowl occurred. On the right, the location of Parque da Liberdade in the city of Quirinópolis, Goiás, Brazil.

3,786,026 km² and an estimated population of 51,323 inhabitants (IBGE 2021). At the time of the outbreak, the park waterfowl population consisted of approximately 80 animals, including ducks (*Cairina moschata*), teals (*Anas platyrhynchos*), and geese (*Anser cygnoides*).

In October 2021, the park's lake was repaired, and the water was partially drained, exposing submerged soil and organic matter. The presence of decomposing garbage on the lake shores has also been reported. An epidemiological survey on the mortality of aquatic birds in the park was carried out by the Sanitary Surveillance team of the municipality of Quirinópolis after a complaint of the presence of dead birds in the park at the end of October.

A total of 30 waterfowl died, including 17 ducks (*C. moschata*), 11 teals (*Anas platyrhynchos*), and two geese (*A. cygnoides*). Of these, six birds showed clinical signs of flaccid paralysis of the hind limbs, wings, neck, and eyelids, which progressed to death (Fig.2). The birds were fed on the ground with a mixture of sorghum, corn, and bran, and the lake was the only source of water.

Laboratory diagnosis. Based on the clinical signs and history of lake repair, botulism was suspected. For the detection of botulinum toxin, four 50mL samples of lake water and two 150g samples of feed offered to the birds were collected. Two adult male teals (*Anas platyrhynchos*) that showed clinical signs were necropsied, and liver, stomach content, intestinal content, and serum samples were collected. In the macroscopic examination, the birds had a healthy nutritional status, and no macroscopic lesions were observed at necropsy.

The samples (liver, stomach content, intestinal content, and serum) collected from the two teals and the water samples were processed and analyzed for botulinum toxin through the standard bioassay technique in mice at the Laboratory of Bacteriology at the "Escolha de Veterinária e Zootecnia" (School of Veterinary and Animal Science) at the "Universidade Federal de Goiás" (UFG). Samples were inoculated (0.5mL) intraperitoneally into mice with body weights ranging from 20 to 25 grams, according to the procedures described by Smith (1977). Meanwhile, specimens from the same samples were heated at 85°C for 20 minutes and inoculated into additional mice, which remained under observation for seven days. The typing of positive samples in the mice bioassay was performed by means of seroneutralization (Smith 1977) with botulinum antitoxin types C and D. Bioassay technique in mice was approved by the Ethics Committee on Animal Use of UFG (Protocol No. 024/20).


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Fig. 2. Three birds, from Parque da Liberdade, showing clinical signs of flaccid paralysis.

Additionally, water and feed samples were seeded in Wright's medium and incubated at 37°C for 5 days for indirect detection of *Clostridium botulinum* spores. Then, the mice were inoculated with the supernatant (0.5mL) and monitored for seven days. The presence of *C. botulinum* types C and D in samples of lake water and feed supernatant seeded in Wright's medium was evaluated by polymerase chain reaction (PCR) with the use of specific primers (Prévot et al. 2007). Amplifications were performed in 20µl volumes containing 11.5µl water for PCR, 1µl Taq Solis BioDyne (Solis BioDyne, Estonia), 1µl of each primer pair (Invitrogen, Brazil and 2.5µl of the sample. This amplification was performed in a PCR thermocycler (Swift™ MaxPro Thermal Cycler, Esco Healthcare Pt Singapore) programmed for one cycle at 95°C for 5 minutes, and 40 cycles at 94°C for 30 seconds, an annealing step at 55°C for 30 seconds, and an extension step at 72°C for 30 seconds, and a final extension of 10 minutes at 72°C (Prévot et al. 2007). PCR amplification products were subjected to electrophoresis on 1% agarose gel and as positive controls were used DNA samples of reference strains.

RESULTS

Botulinum toxin was identified in one of the four water samples collected from the lake, and in the intestinal content of one of the necropsied teals (Table 1). Thus, mice inoculated with these samples developed paralysis and dyspnea ("wasp waist") and died within 12 to 24 hours after inoculation. None of the mice inoculated with the heat-treated samples showed any clinical symptoms. In the feed samples, the presence of botulinum toxin or even the presence of the bacteria through the culture was not detected (Table 1).

Using the serum neutralization technique in mice, botulinum toxin type C was detected in a water sample and an intestinal contents sample (Table 1). By PCR, the presence of *Clostridium botulinum* type C was identified in two water samples collected from the lake (Table 1, Fig.3).

Table 1. Results of the direct search for botulinum toxin in biological samples of aquatic birds, water and feed by the mouse bioassay and the identification of *Clostridium botulinum* in water and feed samples by PCR

Tested sample (N*)	Mouse bioassay (N**)	Seroneutralization in mice	PCR (N***)
Water (4)	Positive (1)	Botulinum toxin type C	*Clostridium botulinum* type C (2)
Feed (2)	Negative	-	Negative
Intestinal content (2)	Positive (1)	Botulinum toxin type C	Unperformed
Stomach content (2)	Negative	-	Unperformed
Liver tissue (2)	Negative	-	Unperformed
Serum (2)	Negative	-	Unperformed

N* = number of samples tested, N** = number of positive samples for the detection of botulinum toxin, N*** = number of positive samples for the identification of *Clostridium botulinum*.

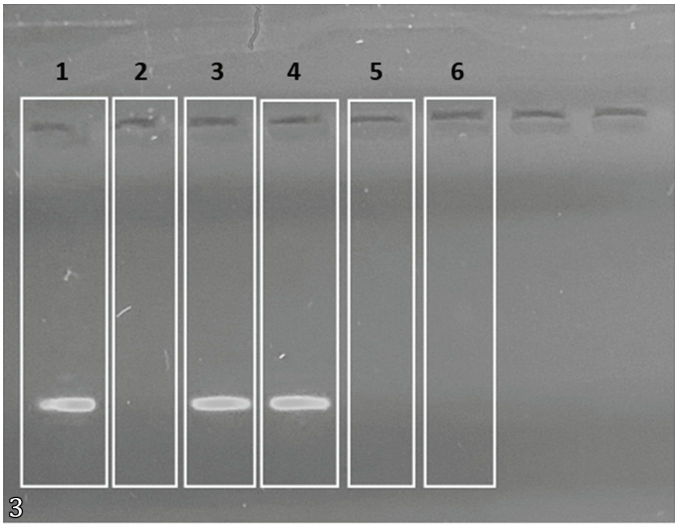

Fig. 3. *Clostridium botulinum* type C identified by PCR in two water samples collected from the lake of Parque da Liberdade, Quirinópolis, Goiás, Brazil. Positive control type C *Clostridium botulinum* (1), negative control (2), water sample 1: positive result (3), water sample 2: positive result (4), water sample 3: negative result (5), water sample 4: negative result (6).

DISCUSSION

The diagnosis of botulism is based on the clinical signs presented by the affected animals, by the history of a rapid increase in mortality of animals with flaccid paralysis, in the absence of lesions in the *post mortem* examination and in the laboratory confirmation (Uzal et al. 2016). Thus, the reported clinical observations and epidemiological data reinforced the suspicion of botulism as a cause of mortality in the park's birds. However, a definitive diagnosis of botulism must be based on the detection and classification of botulinum toxin(s) in samples collected from suspected animals or in water and food samples (Rocke & Bollinger 2007, Uzal et al. 2016).

The mouse bioassay is specific and sensitive and is still considered the gold standard for the detection and identification of botulinum toxins. However, false negative results are common because some species are more sensitive to botulinum toxin than mice or because there is no more toxin circulating at the time of collection. In addition, the bioassay is a laborious and time-consuming method, presents a high cost, there is a need for its own facilities to maintain mice and ethical issues (Uzal et al. 2016).

The results of the bioassay in mice indicated the presence of botulinum toxin in the water collected from the lake at the park inhabited by the birds and in the intestinal content of one of the teals that showed clinical signs of flaccid paralysis. Through serum neutralization in mice, it was possible to identify botulinum toxin type C in these samples. These laboratory results, as well as the clinical and epidemiological history, confirmed the diagnosis of botulism type C as the cause of waterfowl mortality in the park.

Conditions considered favorable for botulism outbreaks in waterfowl include large amounts of protein-rich organic matter, little available oxygen, high temperatures, and shallow water. The drainage of natural and artificial aquatic environments can favor the multiplication of *Clostridium botulinum* and the occurrence of outbreaks, as it predisposes the accumulation of organic matter and decreased oxygen availability (Lima et al. 2020). Botulism outbreaks usually occur during the warmest months of the year (Rocke & Bollinger 2007) and October is considered the warmest month of the year in the city of Quirinópolis, Goiás, which coincides with the time of the outbreak (Weather Spark 2022).

Thus, the recent maintenance history of the lake, associated with the presence of organic matter and favorable climatic characteristics, may have contributed to the establishment of an environment conducive to *Clostridium botulinum* multiplication and the production of botulinum toxin, culminating in the occurrence of botulism in birds that drank the lake water. All bird species involved in the outbreak are part of the Anatidae family and are considered filtering animals; that is, they have the ability to remove food from water through a filtering process (Carboneras 1992). Thus, the detection of bacteria and botulinum toxin type C (Table 1) in water samples reinforces the suspicion that the contamination origin was the lake environment itself and that the water was the vehicle for intoxication. However, sediment samples were not collected and analyzed, which may have provided important complementary information as evidence of such suspicion.

In Goiás, waterborne botulism in cattle is associated with environmental contamination by bacteria, epidemiological conditions that culminate in the accumulation of decomposing organic matter, and variations in water levels in the wells and its use in watering animals (Souza et al. 2011). In birds, several risk factors for water intoxication have been identified, such as the presence of organic matter in the water source and stagnant water reservoirs, such as puddles and ponds. These water sources are considered more problematic in the dry season because of the reduction in water levels, which can lead to the exposure of decomposing organic matter thereby increasing the risk of intoxication, since the toxins are more concentrated (Galvin et al. 1985, Lobato et al. 2013, Anza et al. 2016). Thus, these scenarios and some of the variables were probably present at the time of the botulism outbreak in birds in Quirinópolis.

Botulism is a disease characterized by high mortality and lethality. Among approximately 80 birds residing in the park, 30 died, resulting in a total mortality rate of 37.5%: 17 ducks (56.6%), 11 teals (36.6%), and two geese (6.6%). Several episodes involving mortality of different bird species by botulism in different epidemiological situations have already been described. In poultry, outbreaks of botulism associated with the consumption of fly larvae and decaying organic matter have been described in turkey (*Meleagris gallopavo*) and chicken (*Gallus domesticus*) farms, with high mortality (Lobato et al. 2009, Alves et al. 2013).

Outbreaks of water botulism have been described in chickens (*G. domesticus*), ducks (*Anas platyrhynchos*), guinea hens (*Numida meleagris*), teals (*Anas querquedula*), and turkeys (*Meleagris* sp.) that ingested water contaminated with organic matter or from sewage systems (Olinda et al. 2015, Quevedo et al. 2022). In the present study, botulinum toxin was detected in the water of the lake where the birds lived and fed.

Lima et al. (2020) reported an outbreak of type C botulism in wild and migratory waterfowl at the Sobradinho Dam. At that time, the level of the dam was low, which caused the formation of shallow and isolated lakes with large amounts of decomposing organic matter, where dead or weakened birds were found. In the present study, very similar epidemiological situations occurred in the lake at Parque da Liberdade in Quirinópolis, causing waterfowl mortality.

Outbreaks with high mortality rates in birds may be related to botulism, West Nile virus, and other arboviruses, avian influenza, species of the genus *Flavivirus*, and heavy metal poisoning (Jones et al. 1983, Scaramozzino et al. 2001, Spackman et al. 2002, Manarolla et al. 2010, Wlodarczyk et al. 2014). In the present outbreak, the presence of birds with clinical signs of flaccid paralysis of the limbs, wings, and neck helped to direct the suspicion of botulism, which was confirmed by the detection of botulinum toxin in samples collected from diseased birds and lake water.

In some countries, experimental immunization against botulinum toxin in birds as a preventive measure has shown positive results in reducing mortality in outbreaks (Martinez & Wobeser 1999, Rocke et al. 2000, Arimitsu et al. 2004). However, there are no reports on immunization of birds with clostridial vaccines in Brazil. Thus, preventive measures aimed at preventing new outbreaks resulting from the ingestion of botulinum toxin represent a challenge in the management of bird populations in urban aquatic environments. Measures such as monitoring the presence of carcasses, garbage, decomposing organic matter, and standing water represent some possible actions that can be established in urban areas.

CONCLUSION

Based on clinical signs, epidemiological data, and laboratory results, waterborne botulism type C was diagnosed as the cause of mortality in free-ranging waterfowl at Parque da Liberdade in Quirinópolis, Goiás, Brazil.

Funding.- This study has not received any specific grants or funding from funding agencies in the public, commercial or not-for-profit sectors.

Conflict of interest statement.- The authors declare that there are no conflicts of interest.

REFERENCES

Alves G.G., Silva R.O.S., Pires P.S., Salvarani F.M., Oliveira Júnior C.A., Souza G.X.W., Santos F.C.M., Caldas R.P., Assis R.A. & Lobato F.C.F. 2013. Surto de botulismo tipo C em frangos na cidade de Pancas, Espírito Santo, Brasil. Semina, Ciências Agrárias 34(1):355-358. <https://dx.doi.org/10.5433/1679-0359.2013v34n1p355>

Anza I., Vidal D., Feliu J., Crespo E. & Mateo R. 2016. Differences in the vulnerability of waterbird species to botulism outbreaks in Mediterranean wetlands: an assessment of ecological and physiological factors. Appl. Environ. Microbiol. 82(10):3092-3099. <https://dx.doi.org/10.1128/AEM.00119-16> <PMid:27016572>

Arimitsu H., Lee J.C., Sakaguchi Y., Hayakawa Y., Hayashi M., Nakaura M., Takai H., Lin S.N., Mukamoto M., Murphy T. & Oguma K. 2004. Vaccination with recombinant whole heavy chain fragments of *Clostridium botulinum* Type C and D neurotoxins. Clin. Diagn. Lab. Immunol. 11(3):496-502. <https://dx.doi.org/10.1128/CDLI.11.3.496-502.2004> <PMid:15138174>

Badagliacca P., Pomilio F., Auricchio B., Sperandii A.F., Provvido A., Ventura M., Migliorati G., Caudullo M., Morelli D. & Anniballi F. 2018. Type C/D botulism in the waterfowl in an urban park in Italy. Anaerobe 54:72-74. <https://dx.doi.org/10.1016/j.anaerobe.2018.07.010> <PMid:30118893>

Barash J.R. & Arnon S.S. 2014. A novel strain of *Clostridium botulinum* that produces type B and type H botulinum toxins. J. Infect. Dis. 209(2):183-191. <https://dx.doi.org/10.1093/infdis/jit449> <PMid:24106296>

Bengston I.A. 1922. Preliminary note on a toxin-producing anaerobe isolated from the larvae of *Lucilia caesar*. Publ. Health Rep. 37(4):164-170. <https://dx.doi.org/10.2307/4576258>

Burke G.S. 1919. Notes on *Bacillus botulinus.* J. Bacteriol. 4(5):555-570.1. <https://dx.doi.org/10.1128/jb.4.5.555-570.1.1919> <PMid:16558852>

Carboneras C. 1992. Family Anatidae (ducks, geese and swans), p.536-629. In: Hoyo J., Elliott A. & Sargatal J. (Eds), Handbook of Birds of the World. Ostrich to Ducks. Vol.1. Lynx Edicions, Barcelona.

Chipault J.G., White C.L., Blehert D.S., Jennings S.K. & Strom S.M. 2015. Avian botulism type E in waterbirds of Lake Michigan, 2010-2013. J. Great Lakes Res. 41(2):659-664. <https://dx.doi.org/10.1016/j.jglr.2015.03.021>

Galvin J.W., Hollier T.J., Bodinnar K.D. & Bunn C.M. 1985. An outbreak of botulism in wild waterbirds in southern Australia. J. Wildl. Dis. 21(4):347-350. <https://dx.doi.org/10.7589/0090-3558-21.4.347> <PMid:4078968>

Gimenez D.F. & Ciccareli A.S.S. 1970. Another type of *Clostridium botulinum*. Zentralbl. Bakteriol. Orig. 215(2):221-224. <PMid:4922309>

Gunnison J.B. & Meyer K.F. 1929. South African cultures of *Clostridium botulinum* and *Parabotulinum.* XXXVII: with a description of *C. botulinum* type D, N.SP. J. Infect. Dis. 45(2):106-118. <https://dx.doi.org/10.1093/infdis/45.2.106>

Gunnison J.B., Cummings J.R. & Meyer K.F. 1936. *Clostridium botulinum* type E. Exp. Biol. Med. 35(2):270-280. <https://dx.doi.org/10.3181%2F00379727-35-8938P>

IBGE 2021. Cidades e Estados: Quirinópolis, Goiás. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro. Available at <https://cidades.ibge.gov.br/brasil/go/quirinopolis/panorama> Accessed on Apr. 1, 2022.

Jones L.M., Both N.H. & McDonald L.E. 1983. Farmacologia e Terapêutica em Veterinária. 4rd ed. Guanabara-Koogan, Rio de Janeiro, p.33-1000.

Lima P.C., Dutra I.S., Araújo F.A.A., Lustosa R., Zeppelini C.G. & Franke C.R. 2020. First record of mass wild waterfowl mortality due to *Clostridium botulinum* in Brazilian semiarid. An. Acad. Bras. Ciênc. 92(1):e20180370. <https://dx.doi.org/10.1590/0001-3765202020180370>

Linares J.A., Walker R.L., Bickford A.A., Cooper G.L. & Charlton B.R. 1994. An outbreak of type C botulism in pheasants and subsequent occurrence in chicken. J. Vet. Diagn. Invest. 6(2):272-273. <https://dx.doi.org/10.1177/104063879400600225> <PMid:8068766>

Lobato F.C.F., Salvarani F.M., Gonçalves L.A., Pires P.S., Silva R.O.S., Alves G.G., Neves M., Oliveira Júnior C.A. & Pereira P.L.L. 2013. Clostridioses dos animais de produção. Vet. Zootec. 20:29-48.

Lobato F.C.F., Salvarani F.M., Silva R.O.S., Assis R.A., Lago L.A., Carvalho Filho M.B. & Martins N.R.S. 2009. Botulismo tipo C em perus em Minas Gerais, Brasil. Ciência Rural 39(1):272-274. <https://dx.doi.org/10.1590/S0103-84782008005000048>

Manarolla G., Bakonyi T., Gallazzi D., Crosta L., Weissenböck H., Dorrestein G.M. & Nowotny N. 2010. Usutu virus in wild birds in northern Italy. Vet. Microbiol. 141(1/2):159-163. <https://dx.doi.org/10.1016/j.vetmic.2009.07.036>

Martinez R. & Wobeser G. 1999. Immunization of ducks for type C botulism. J. Wildl. Dis. 35(4):710-715. <https://doi.org/10.7589/0090-3558-35.4.710> <PMid:10574530>

Moller V. & Scheibel I. 1960. Preliminary report on the isolation of an apparently new type of *Cl. botulinum*. Acta Pathol. Microbiol. Scand. 48:40. <https://dx.doi.org/10.1111/j.1699-0463.1960.tb04741.x> <PMid:14423425>

Morner T., Obendorf D.L., Artois M. & Woodford M.H. 2002. Surveillance and monitoring of wildlife diseases. Rev. Sci. Tech. 21(1):67-76. <https://dx.doi.org/10.20506/rst.21.1.1321> <PMid:11974631>

Neimanis A., Widén D.G., Leighton F., Bollinger T., Rocke T. & Morner T. 2007. An outbreak of type C botulism in herring gulls (*Larys argentatus*) in southeastern Sweden. J. Wildl. Dis. 43(3):327-336. <https://dx.doi.org/10.7589/0090-3558-43.3.327> <PMid:17699071>

Olinda R.G., Gois R.C.S., Silva R.O.S., Caldas R.P., Lobato F.C.F. & Batista J.S. 2015. Surto de botulismo tipo C em aves domésticas no semiárido do Nordeste, Brasil. Revta Bras. Ciênc. Vet. 22(3/4):157-159. <https://dx.doi.org/10.4322/rbcv.2016.006>

Prévot V., Tweepenninckx F., Van Nerom E., Linden A., Content J. & Kimpe A. 2007. Optimization of polymerase chain reaction for detection of *Clostridium botulinum* type C and D in bovine samples. Zoonoses Publ. Health 54(8):320-327. <https://dx.doi.org/10.1111/j.1863-2378.2007.01070.x> <PMid:17894643>

Quevedo L.S., Schmitt S.E., Withoeft J.A., Cristo T.G., Figueiredo C.A., Santana J.A., Silva R.O.S. & Casagrande R.A. 2022. Surto de botulismo tipo C em aves de subsistência em Santa Catarina, Brasil. Acta Sci. Vet. 50(Supl.1):753. <https://dx.doi.org/10.22456/1679-9216.118874>

Rocke T.E. & Bollinger T.K. 2007. Avian botulism, p.377-416. In: Thomas N.J. & Atkinson C.T. (Eds), Infectious Diseases of Wild Birds. Blackwell Publishing, Oxford.

Rocke T.E. 2006. The global importance of avian botulism, p.422-426. In: Boere G.C., Galbraith C.A. & Stroud D.A. (Eds), Waterbirds Around the World. The Stationery Office, Edinburgh.

Rocke T.E., Samuel M.D., Swift P.K. & Yarris G.S. 2000. Efficacy of a type C botulism vaccine in green-winged teal. J. Wildl. Dis. 36(3):489-493. <https://dx.doi.org/10.7589/0090-3558-36.3.489> <PMid:10941734>

Rogers K.H., Viera O.G., Uzal F.A., Peronne L. & Mete A. 2021. Mortality of western gulls (*Larus occidentalis*) associated with botulism type A in Coastal southern California, USA. J. Wildl. Dis. 57(3):657-661. <https://dx.doi.org/10.7589/jwd-d-20-00153> <PMid:33956128>

Rosciano N.G., Cossa N.A., Farace M.I., Ojeda V. & Seijas S. 2021. Outbreak of type C botulism in aquatic birds on the Nahuel Huapi Lake and national park area, Argentina. Hornero 36(1):83-88.

Scaramozzino N., Crance J.M., Jouan A., DeBriel D.A., Stoll F. & Garin D. 2001. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. J. Clin. Microbiol. 39(5):1922-1927. <https://dx.doi.org/10.1128/JCM.39.5.1922-1927.2001> <PMid:11326014>

Scarcelli E. & Piatti R.M. 2002. Patógenos emergentes relacionados à contaminação de alimentos de origem animal. Inst. Biol. 64(2):123-127.

Smith L.D.S. 1977. Botulism: the organism, its toxins, the disease. Charles C. Thomas Publisher, Illinois. 236p.

Souza A.M., Filho Dias F.C., Dutra I.S., Marques D.F., Silva S.H., Souza J.A., Santos P.M., Godoi W.P. & Gomes R.O.M. 2011. Ocorrência de *Clostridium botulinum* tipos C e D em criatórios de bovinos no município de Cocalinho, Vale do Araguaia, Mato Grosso. Vet. Zootec. 18(4 Supl.3):831-834.

Spackman E., Senne D.A., Myers T.J., Bulaga L.L., Garber L.P., Perdue M.L., Lohman H., Daum L.T. & Suarez D.L. 2002. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. J. Clin. Microbiol. 40(9):3256-3260. <https://dx.doi.org/10.1128/JCM.40.9.3256-3260.2002> <PMid:12202562>

Trampel D.W., Smith S.R. & Rocke T.E. 2005. Toxicoinfectious botulism in commercial caponized chickens. Avian Dis. 49(2):301-303. <https://dx.doi.org/10.1637/7330-011805> <PMid:16094840>

Uzal F.A., Songer J.G., Prescott J.F. & Popoff M.R. 2016. Clostridial Diseases of Animals. Wiley Blackwell, New Jersey, p.332. <https://dx.doi.org/10.1002/9781118728291>

Weather Spark 2022. Clima e condições meteorológicas médias em Quirinópolis no ano todo. Weather Spark. Available at <https://pt.weatherspark.com/y/29858/Clima-caracter%C3%ADstico-em-Quirin%C3%B3polis-Brasil-durante-o-ano> Accessed on Apr. 5, 2022.

Wlodarczyk R., Minias P., Kukier E., Grenda T., Śmietanka K. & Janiszewski T. 2014. The first case of a major avian type C botulism outbreak in Poland. Avian Dis. 58(3):488-490. <https://dx.doi.org/10.1637/10669-091913-Case.1> <PMid:25518447>